on the upper surfaces of the leaves by shaking abundantly rusted plants over them. A period of incubation of 48 hours in a saturated atmosphere was found to be satisfactory. When infection had reached its maximum development (14–18 days after inoculation), notes on the reactions were taken according to the following infection types:

I *Host immune*: no trace of mycelial invasion.

R+ *Host very resistant*: small hypersensitive flecks shown.

R *Host resistant*: hypersensitive flecks shown on the upper surface of the leaf blade; corresponding development of minute pustules on the lower leaf surface.

R– *Host moderately resistant*: light-brown necrotic spotting of the upper surface of the leaf blade; corresponding development of small to medium pustules on the lower leaf surface.

S– *Host moderately susceptible*: pustules on the upper surface medium in size, surrounded by a chlorotic halo; corresponding development of pustules medium in size on the lower surface.

S *Host susceptible*: pustules on both leaf surfaces medium in size, coalescence infrequent; no visible expression of antagonism between host and parasite.

S+ *Host very susceptible*: pustules on both surfaces large and confluent; often associated with crinkling of the leaf tissue.

TABLE 3.

Origin of Isolates Used in Studies of the Species Host Ranges of Australian Clover Rusts.

Rust.	Origin of Isolate.
U. t. trifolii-repentis	Grafton, N.S.W.
U. t. (*glomerati*)	Gosford, N.S.W.
U. t. fallens	Castle Hill, N.S.W.
U. t. subterranei r. A	Castle Hill, N.S.W.
U. t. subterranei r. B	Bombala, N.S.W.
U. f. (*repentis*)	Sydney, N.S.W.
U. f. (*fragiferi*)	Melbourne, Vic.

The series of reaction types based on a scale of 0–4, so commonly employed in cereal rust work (Stakman and Levine, 1922), was not found satisfactory here. The symbols I, R, S, were used to avoid confusion with the more common numerical designations. No host reaction comparable with the "mesothetic" reaction was encountered.

Results and Discussion.

In Tables 4 and 5 are given the reactions of species of *Trifolium* and related genera to the Australian clover rusts under test, and the number of strains tested within each host species. The information concerning *U. t. trifolii-repentis* and *U. t.* (*glomerati*) was limited by the small amount of uredospore inoculum produced.

In most instances, the reactions of the plants within a given strain showed little variation, but a few strains contained plants showing marked differences in reaction to a given rust isolate, and for these the range in reaction is given. In general, different strains of the one host species behaved similarly in their reactions to a given rust isolate, though there were exceptions. Five strains of *T. glomeratum* were susceptible to *U. t. fallens*, whereas the remaining nine strains were very resistant. One strain of *T. glomeratum* was very resistant to both races of *U. t. subterranei*, while the remaining 13 were susceptible. The reaction of one strain of *T. incarnatum* to *U. t. fallens* (R+) also differed from that of the other six strains (S). The two strains of *T. resupinatum* tested gave contrasting reactions to infection by *U. t. fallens*.

The following conclusions can be drawn from the experimental results recorded:

1. The two physiologic races of *U. t. subterranei* induce almost identical reactions in all host species.

TABLE 4.

Reactions of Species of Trifolium *and Related Genera to Australian Clover Rusts.*

Species.	Number of Strains.	Reaction Induced by				
		U. f. (*repentis*).	*U. f.* (*fragiferi*).	*U. t. fallens.*	*U. t. subterranei.*	
					r. A.	r. B.
Trifolium agarium L.	2	I	S−	S−	S+	S+
T. alexandrinum L.	3	I	I	R+	R	R
T. angustifolium L.	2	I	I	S+	S+	S+
T. arvense L.	2	I	I	I	I	I
T. balansae Boissier	2	S+	S+	S+	S+	S+
T. carolinianum Michx.	1	I		I	I	
T. cernuum Brot.	2	S	S	S+	S+	S+
T. dubium Sibth.	3	I	I	I	I	I
T. fragiferum L.	9	S	S	R+	S	S
T. globosum L.	2	I	I	R	I	I
T. glomeratum L.	14	S+	S+	R+	S	S
T. hirtum All.	2	S	S−	R+ & S−	S+	S+
T. hybridum L.	6	S	S−	I	S	S
T. incarnatum L.	7	S	S−	S	S	S+
T. lappaceum L.	1	I		I	I	
T. lupinaster L.	1			R+	I	
T. maritimum Huds.	1	I	I	S−	S−	S− & R−
T. medium L.	1	I		I	R+	
T. nigrescens Viv.	2	S	S	I	S+	S+
T. pallidum Waldst. and Kit.	2	I	I	S+	I	R+
T. pratense L.	3	S−	S	S+ & R+	S	S
T. procumbens L.	1	I		I	R+	
T. reflexum L.	1	R+		R+	R+	
T. repens L.	10	S+	I	I	I	I
T. resupinatum L.	2	S	S	S+ & R+	S+	S+
T. scabrum L.	1	S−	S−	S	S+	S
T. squarrosum Bieb.	1	I	S+	S	S+	S+
T. stellatum L.	1				R+	
T. striatum L.	1	S	S	S+	S+	S+
T. subrotundum Steud. and Hochst.	1	I		I	S+	
T. subterraneum L. (Mt. Barker)	1	I	I	I	S+	S+
T. tomentosum L.	1	S			I	
T. tridentatum Lindl.	2	I	I	I	I	R+
T. wormskjoldi Lehm.	1			I	I	
T. xerocephalum Fenzl.	1				I	
Lotus americanus Bisch.	1					I
L. corniculatus L.	1			I	I	I
L. hispidus Desf.	1			I	I	I
L. major Sm.	1					I
Medicago sativa L.	3	I		I	R+	I
Melilotus alba Desr.	2	I		I	I	R+
M. officinalis Lamk.	1					I
Trigonella suavissima Lindl.	1				S	
Vicia atropurpurea Desf.	1					I

2. Only three species, viz., *T. agarium*, *T. repens*, and *T. squarrosum*, differ appreciably in their reactions to *U. f.* (*repentis*) and *U. f.* (*fragiferi*).
3. The host ranges of *U. t. fallens* and *U. t. subterranei* differ markedly, 10 of the 35 species of *Trifolium* giving contrasting reactions.
4. The host ranges of both *U. t. fallens* and *U. t. subterranei* differ in many respects from those of *U. f.* (*repentis*) and *U. f.* (*fragiferi*).
5. *U. t. subterranei* is able to attack a species of *Trigonella*. In no other instance has the host range of a clover rust extended beyond the genus *Trifolium*.

U. t. trifolii-repentis and *U. t.* (*glomerati*) cannot be compared owing to the meagre information available, but the inability of the latter to attack the two strains of *T. repens* on which it was tested is interesting and might be carried further.

The host ranges of *U. t. hybridi* (Kobel, 1920; Mains, 1935) and *U. t. subterranei* show some similarity, the former being able to attack *T. subterraneum* and the latter *T. hybridum*. However, the rusts give contrasting reactions on *T. agarium*, *T. arvense*, *T. resupinatum* and *T. pratense*. Of particular significance is the susceptibility of seedlings of *T. pratense* to *U. t. subterranei*; extensive cross-inoculation studies (Liro, 1906; Davis, 1924) have demonstrated the resistance of *T. pratense* to attack by *U. t. hybridi*.

The Australian rust, *U. f.* (*repentis*), has a host range which is similar to that of the macrocyclic form on the same host species, *U. t. trifolii-repentis*, as given by Kobel and Mains. However, the microcyclic form is able to attack both *T. pratense* and *T. hybridum*; these two species are recorded by Liro (1906), Davis (1924), Kobel (1920) and Mains (1935) as resistant to *U. t. trifolii-repentis*.

TABLE 5.

Reactions of Species of Trifolium *to Australian Clover Rusts.*

Species.	Number of Strains.	Reaction Induced by	
		U. t. trifolii-repentis.	*U. t.* (*glomerati*).
T. glomeratum L. ..	4	S	S
T. pratense L.	1	I	I
T. repens L.	2	S	I
T. subterraneum L. ..	1	I	I

Physiologic Specialization of Subterranean Clover Rust.

Introduction.

Leaf rust (*U. t. subterranei*) appears to be the most destructive of the diseases of subterranean clover in Australia. During favourable seasons, serious losses in grazing potential may result owing to the widespread cultivation of susceptible varieties. Resistant varieties are available, but in general their yielding capacity is not high. The incorporation of rust resistance into susceptible but prolific commercial varieties would therefore be an important step in reducing such losses.

Continued freedom from the disease, however, must be based on an understanding of the physiologic specialization shown by the pathogen. No evidence of specialization in *U. t. subterranei* has yet been recorded. Little information is available on the reactions of subterranean clover varieties to rust, and the observations recorded by different workers are not completely concordant.

Accordingly, the present study was undertaken with the following objectives:

1. To search for evidence of specialization in the pathogen;
2. To determine the distribution in Australia of any physiologic races detected;
3. To determine the reactions of all available varieties to each physiologic race.

Review of Literature.

Australian, American and European varieties of subterranean clover have been classified by Aitken and Drake (1941). Observations were made of more than 200 samples grown year after year at Burnley Gardens, Melbourne, and more than 50 varieties differentiated, each exhibiting an extreme stability of type. Levy and Gorman (1937) classified 25 varieties for resistance to leaf rust during plot trials at Palmerston North, New Zealand. Radel (1935), in discussing Tasmanian field trials, mentioned the relative resistance to leaf rust of several varieties. Loftus Hills (1942) has summarized the

results of field observations on the reactions to leaf rust of 24 varieties grown at Moss Vale, N.S.W., and Canberra, A.C.T. The observations at the two localities did not in all instances agree, and discrepancies were found between these results and those recorded by Radel, and Levy and Gorman.

A satisfactory technique for the hybridization of subterranean clover varieties has been developed by McMillan (1937). From field observations on the F2 generation of a cross between Mt. Barker and the immune early-maturing variety Mulwala, Loftus Hills (1944) concluded that resistance to leaf rust was an inherited character in which susceptibility was dominant, and that a variety could probably be evolved which would combine the desirable agronomic characters of Mt. Barker with the rust resistance of Mulwala.

Materials and Methods.

Seed of 70 varieties of subterranean clover was obtained from the Pasture Research Station, Burnley; the Division of Plant Industry, C.S.I.R.O., Canberra; and the Department of Agriculture, N.S.W. In some instances seed of a given variety was received from more than one source, and each seed sample has been studied individually.

TABLE 6.

Reactions of Selected Varieties of T. subterraneum *to Three Physiologic Races of* U. t. subterranei.

Test Variety.	Reaction to Physiologic Race.		
	"A."	"B."	"C."
Bacchus Marsh	R & S	S	S
Clare	R	S−	R
Dwalganup	R−	S	R−
Madrid..	S	S+	S
Mt. Barker	S+	S+	S+
Mulwala	I	I	I
Tallarook	S	S	S
Yarloop	R+	S+	R−

Isolates of *U. t. subterranei* have been collected from 27 localities throughout the subterranean clover areas of Australia. Cultures of the rust isolates were maintained on plants of the variety from which they were collected. A technique was developed involving the use of seedlings grown in 6″ × 1″ glass tubes stoppered with loose cotton wool plugs, a method most useful for the initiation of cultures from specimens collected during surveys of country areas. A large number of cultures was thus maintained, without fear of mixing, until glasshouse space became available. In the bottom of each tube were placed a few small pebbles covered with a piece of cotton wool, to drain excess water from the sifted soil above. Watering was necessary only according to the amount of water visible in the bottom of the tubes. The roots of the seedlings were kept in darkness by means of a strip of brown paper around the base of each tube.

Because of the manual work involved, seedlings grown under tube conditions were unsatisfactory for the general testing work, and the method described in the previous section was used. The testing period was from May, 1951, to February, 1952.

Results.

A set of eight subterranean clover varieties was selected arbitrarily for the comparison of isolates of *U. t. subterranei* (Table 6). As each isolate came to hand it was built up on susceptible plants and tested on the selected varieties. The individual plant reactions within each variety except Bacchus Marsh were completely uniform, and 15–20 seedlings of a given variety provided a most satisfactory basis for comparative studies.

By this means three physiologic races of the pathogen were differentiated. The reactions of the selected host varieties to the races, recorded under uniform environmental conditions, are given in Table 6. The races are most easily recognized by use of the variety Yarloop. A summary is given in Table 7 of the number of times each race has been isolated from material collected in each State. Table 8 lists the localities within each State from which isolates have been collected, together with racial designations.

From a breeding viewpoint, it is important to know not only the distribution of each physiologic race, but also the relative susceptibility of all available host varieties.

TABLE 7.

Frequency of Isolation of Physiologic Races of U. t. subterranei *from Material Collected in the States of Australia.*

Race.	Number of Isolations in Each State.					Total.
	N.S.W.	Vic.	Tas.	S.A.	W.A.	
A	16	1	1	1	5	24
B	1	4				5
C	1					1
Totals ..	18	5	1	1	5	30

TABLE 8.

Localities Throughout Australia from which Isolates of U. t. subterranei *have been Obtained.*

Locality.	Race.	Locality.	Race.
Glen Innes, N.S.W.	A	Bombala, N.S.W.	B
Wingham, N.S.W.	A	Lang Lang, Vic.	B
Oxley Island, N.S.W. ..	A	South Gippsland, Vic. ..	B
Gloucester, N.S.W.	A	Burnley, Vic.	B
Tuncurry, N.S.W.	C	Box Hill, Vic.	B
Maitland, N.S.W.	A	Moorooduc, Vic.	A
Capertee, N.S.W.	A	Cressy, Tas.	A
Castle Hill, N.S.W.	A	Glen Osmond, S.A. ..	A
Goulburn, N.S.W.	A	Esperance, W.A.	A
Canberra, N.S.W.	A	Manjimup, W.A.	A
Leeton (M.I.A.), N.S.W. ..	A	Marybrook, W.A.	A
Griffith (M.I.A.), N.S.W. ..	A	Waterloo, W.A.	A
Bodalla, N.S.W.	A	Bullsbrook, W.A. ..	A
Bega, N.S.W.	A		

The reactions of 70 varieties to race A from Castle Hill, N.S.W., race A from Manjimup, W.A., race B from Bombala, N.S.W., and race B from Box Hill, Vic., were therefore determined. The results are summarized in Table 9. With the varieties listed in Table 6, the seedling reactions under glasshouse conditions correspond to those of three-months-old plants raised out of doors.

Discussion.

Physiologic specialization in *U. t. subterranei* must influence the problem of breeding rust resistant varieties. On the basis of the evidence at hand, race B appears to be restricted to Victoria and southern New South Wales, but it must be considered capable of spreading to all subterranean clover areas in Australia. Presumably the race has

TABLE 9.

Reactions of Varieties of T. subterraneum *to Physiologic Races of* U. t. subterranei.

Variety.	Number of Samples.	Reaction to Physiologic Race.			
		"A." N.S.W.	"A." W.A.	"B." N.S.W.	"B." Vic.
Amber-seeded Mt. Barker	1	S	S+	S	S
Bacchus Marsh	5	R & S	R & S−	S	S
Bass	3	R−	R−	S	S
Baulkamaugh North	1	I	I	I	I
Bena	2	S	S	S+	S
Benalla	1	S−	S	S+	S
Berlin	1	R+	R+	S	S
Burnerang	2	S−	S	S+	S+
Burnley	2	S−	S−	S	S
Casterton	2	R	R	S	S
Clare	3	R	R	S−	S−
Cranmore	1	S+	S+	S	S
Daliak	2	R+	R+	S	S
Derrinal	1	R	R+	S	S
Dwalganup	5	R−	R−	S	S
Edenhope	2	S	S	S	S
Flinders	1	R+	R	S	S
Gin Gin	1	R+	R+	S	S
Hexham Hairy Stem	2	R	R	S+	S+
Hexham Smooth Stem	2	R	R	S+	S
Hill's Small	2	R	R−	S	S
Horsham	1	R−	R	S	S
Kilmore	2	R+	R+	S	S
Kyabram	2	S−	S−	S+	S+
Kybybolite	1	S+	S+	S	S
Kyneton	1	R+	R	S	S
Lake Midgeon	2	S	S−	S+	S+
Leige	1	R−	S−	S	S
MacArthur	1	S	S−	S	S
Madrid	3	S	S+	S+	S
Mansfield	2	S−	S−	S+	S+
Merino	1	S−	S	S	S
Milton	2	R−	R	S	S
Mt. Barker	5	S+	S+	S+	S+
Mulwala	2	I	I	I	I
Muresk	1	R	R	S	S
Nangeela	5	R	R	S	S
New Northam Early	1	R−	R	S	S
Northam	1	R−	R−	S	S
Northam B	1	R−		S	
Northam C	1			S−	
Northam D	1			S−	
Orford	1	S+	S+	S	S
Pahantamu	1	R−	R−	S	S
Phillip Island	1	S−	R−	S+	S
Pink Flowered	2	R−	R−	S	S
Port Fairy	1	S	S−	S+	S+
Portugal	1	S+	S+	S+	S+
Portugal A	1	R	R	S	S
Redleaf	1	S	S	S	S
Romsey	1	S−	S−	S	S
Rouen	1	R+	R	S	S
Ruakura Farm	1	R+	R+	S+	S
Ruakura Selection	1	R+	R+	S	S+
Samaria	2	R	R	S	S
Seaton Park	2	S−	S	S	S
Smeaton	2	R	R+	S	S
Springhurst	2	R	R+	S	S
Tallarook	7	S	S	S	S
Turkish	1	R	R	S	S−
Wallendbeen	1	S+	S+	S	S

TABLE 9.—*Continued.*

Reactions of Varieties of T. subterraneum *to Physiologic Races of* U. t. subterranei.—Continued.

Variety.	Number of Samples.	Reaction to Physiologic Race.			
		"A." N.S.W.	"A." W.A.	"B." N.S.W.	"B." Vic.
Wangarrata	1	S−	S−	S+	S+
Wenigup	2	R+	R+	R+	R+
White-seeded Mt. Barker ..	1	S+	S+	S+	S
Williams	1	R+	R	S	S
Wodonga	1	R+	R	S	S
Yabba North	1	R	R	S	S
Yarloop	6	R+	R+	S+	S+
Yea	2	R	R+	S	S

arisen in Victoria during recent years, its greater aggressiveness having resulted in a progressive widening of its distribution.

Breeding programmes in States other than Victoria cannot be based solely on the principle of "field-exposure" to rust attack. Varieties developed by such a method would carry resistance to race A of *U. t. subterranei*, but not necessarily to race B, and might be rendered "field-susceptible" by the subsequent introduction of race B to the area.

Physiologic specialization in *U. t. subterranei* has some significance from a mycological viewpoint as well, being the first recorded instance of specialization within a subspecies of *U. trifolii*.

The available sources of resistance to leaf rust are shown in Table 9. There are 19 varieties which show strong resistance (I or R+) to race A, but only three which are resistant to race B, viz., Baulkamaugh North, Mulwala and Wenigup. Since each of the three varieties shows immunity to both races of the pathogen, its value as a resistant parent in breeding work is obvious.

The host ranges of the isolates of race A from New South Wales and Western Australia are identical; this is also the case with the isolates of race B from New South Wales and Victoria. The series of eight test varieties used in the comparative studies was apparently adequate.

There appears to be no strict correlation of the flowering date of a given host variety to its rust reaction, though the rust incidence under field conditions is no doubt affected by seasonal factors.

Much more must be learned before our knowledge of the physiologic races of *U. t. subterranei* in Australia is complete. An intensive survey of each State to detect all physiologic races, and to provide information about the distribution in space of each, should ideally be made annually. The most urgent question arising from the present investigation concerns the distribution in space and time of race B.

Biometrical Studies of Spore Dimensions.

Literature Review.

Comparative biometrical studies of spore morphology have been recorded for few species of plant rusts. Levine (1923) presented the first statistical information on the spores of subspecies of *Puccinia graminis*, all of which showed significant differences in uredospore, teleutospore, and aecidiospore dimensions when cultured under uniform conditions. A comparative study of the uredospores of eight physiologic races of *P. g. tritici*, made by Levine (1928), detected significant differences in their size and shape. The differences were often found to be similar in magnitude to those between subspecies of *P. graminis*.

Waterhouse (1930) made biometrical studies of the spore morphology of cereal rusts in Australia. Significant differences were shown between subspecies of *P. graminis*, between physiologic races of *P. g. tritici*, and between races of *P. triticina*.

The spore forms of rusts of *Trifolium* spp. have hitherto been characterized by the ranges within which measurements of length and breadth have fallen.

Methods and Material.

To ensure representative spore samples, efforts were made to standardize the conditions under which the rusts developed, and the procedures of spore sampling and measurement. The spores were produced on fully susceptible host plants under glasshouse conditions, and all measurements were made during a period of two months. Closely related rusts were cultured simultaneously.

All rust cultures were initially derived from field collections. Teleutospores of *U. t. subterranei* and *U. t. fallens* could not be induced under glasshouse conditions, and those from field collections were used in these studies.

To ensure the complete maturity of the spore sample, heavily infected leaves of the host were lightly shaken over a large drop of 50% aqueous lactic acid on a slide. After mounting, a constant period was allowed before measurement. All measurements were

TABLE 10.

Dimensions of Teleutospores of Subspecies of U. flectens *and* U. trifoli.

Subspecies.	Teleutospore Dimensions.		
	Mean Length in Microns.	Mean Breadth in Microns.	Mean Ratio.
U. f. (*repentis*)	23·55±0·13	19·01±0·09	1·25±0·009
U. f. (*fragiferi*)	22·90±0·12	18·79±0·09	1·23±0·009
U. t. trifolii-repentis	23·16±0·12	19·16±0·08	1·22±0·008
U. t. subterranei A	23·83±0·15	18·96±0·09	1·27±0·010
U. t. fallens	24·24±0·13	18·22±0·09	1·34±0·011
U. t. (*glomerati*)	25·80±0·19	17·57±0·10	1·49±0·017

made with the same Watson "Service" microscope, and the same combination of ocular and objective used with an ocular micrometer. The same artificial light intensity was used throughout.

To avoid unconscious selection of spores, every spore encountered in passing was considered for measurement, but with teleutospores, and with all uredospores except those of *U. t. fallens*, it was possible to reject for measurement those spores orientated with their long axes at right angles to the plane of the slide, by careful observation of the germpore distribution. The greatest length and breadth were taken for uredospores. For teleutospores, the greatest breadth was taken, and the length was measured from the tip of the hyaline papilla covering the apical pore to the point of attachment of the pedicle. The ratio of length to breadth was calculated for each spore.

The procedure adopted by Levine (1928) for the determination of the size of an adequate spore sample, related to the particular rusts studied, and to the conditions of rust culture, spore sampling and spore measurement was applied, and it was found that a sample of 50 spores, collected and measured in the manner described, was adequate. In the comparative studies, a spore sample of 200 was used; the ability of the resulting statistics to characterize the rusts is thus assured.

Results.

(*a*) Teleutospore comparisons.

In Table 10 the statistics resulting from the measurement of 200 teleutospores of each of the two subspecies of *U. flectens*, and of the four subspecies of *U. trifolii*, are

presented. Teleutospores of race B of *U. t. subterranei* could not be induced under glasshouse conditions, nor were they present in any field collection. Errors given for the means are the standard errors. A summary of the differences between means is given in Table 11. Test statistics are obtained by dividing the difference to be tested by its standard error. For a one-sided *t*-test, a test statistic greater than 2·3 is significant at the ·01 level of probability.

The rusts can be seen to show significant differences in teleutospore length and breadth. Teleutospores of *U. f.* (*repentis*) have a mean length which exceeds that of the teleutospores of *U. f.* (*fragiferi*) by 0·65 ± 0·18 μ. The means obtained for the subspecies of *U. trifolii* vary by as much as 2·64 ± 0·22 μ in length, and 1·59 ± 0·13 μ in breadth.

In Table 10 the subspecies of *U. trifolii* are arranged in order of increasing average length and decreasing average breadth of the teleutospores, showing the consistent negative correlation between the two dimensions. Teleutospores of subspecies of *U. flectens* do not conform to the same pattern.

The non-significant differences between the teleutospores of *U. f.* (*repentis*) and *U. t. trifolii-repentis* are summarized in the last line of Table 11. Such similarity favours theories of a common origin of the two species on *T. repens*.

TABLE 11.

Summary of Differences between Means of Dimensions of Teleutospores of Subspecies of U. flectens *and* U. trifolii.

Rusts Compared.	Length.		Breadth.		Ratio.	
	Difference in Means in Microns.	Test Statistic.	Difference in Means in Microns.	Test Statistic.	Difference in Means.	Test Statistic.
U. f. (*repentis*) and *U. f.* (*fragiferi*)	0·65±0·18	3·6*	0·22±0·13	1·7	0·02±0·013	1·5
U. t. trifolii-repentis and *U. t. subterranei* A	0·67±0·19	3·5*	0·20±0·12	1·7	0·05±0·013	3·8*
U. t. trifolii-repentis and *U. t. fallens*	1·08±0·18	6·0*	0·94±0·12	7·8*	0·12±0·014	8·6*
U. t. trifolii-repentis and *U. t. (glomerati)*	2·64±0·22	12·0*	1·59±0·13	12·2*	0·27±0·019	14·2*
U. t. subterranei A and *U. t. fallens*	0·41±0·20	2·1	0·74±0·13	5·7*	0·07±0·015	4·7*
U. t. subterranei A and *U. t. (glomerati)*	1·97±0·24	8·2*	1·39±0·13	10·7*	0·22±0·020	11·0*
U. t. fallens and *U. t. (glomerati)*	1·56±0·23	6·8*	0·65±0·13	5·0*	0·15±0·020	7·5*
U. f. (*repentis*) and *U. t. trifolii-repentis*	0·39±0·18	2·2	0·15±0·12	1·3	0·03±0·012	2·5*

* Significant at P = 0·01.

(*b*) Uredospore comparisons.

In Table 12 are presented the dimensions of uredospores of subspecies of *U. trifolii* and of races of *U. t. subterranei*. There are considerable differences in uredospore dimensions within the rust species, the subspecies differing by up to 1·54 ± 0·13 μ in length and 2·00 ± 0·11 μ in breadth. Race A of *U. t. subterranei* has a mean uredospore which is 0·54 ± 0·14 μ longer than that of race B. The difference in the average ratio of length to breadth shown by the two races was noticeable from a direct visual comparison of groups of uredospores.

In Table 12 the mean uredospore lengths are arranged in order of increasing magnitude. A negative correlation between length and breadth is apparent among all subspecies except *U. t. fallens*, whose average uredospore is longer and wider than that of any other subspecies. *U. t. fallens* has uredospores with 4–7 scattered germpores, while the other subspecies all have 2–4 equatorial germpores.

The inter-subspecies differences in uredospore dimensions, and the difference in mean length shown by races A and B of *U. t. subterranei*, are of a similar order. Levine (1928) and Waterhouse (1930) record similar properties of cereal rusts. Since physiologic races showing similar morphological differences may occur within other subspecies, the statistics given in Tables 10 and 12 are characteristic only of the entities tested, and not necessarily of the subspecies of which the entities are representative.

TABLE 12.

Dimensions of Uredosporcs of Subspecies of U. trifolii.

Subspecies.	Uredospore Dimensions.		
	Mean Length in Microns.	Mean Breadth in Microns.	Mean Ratio.
U. t. subterranei B	21·85±0·09	20·28±0·07	1·09±0·004
U. t. subterranei A	22·39±0·11	20·25±0·08	1·11±0·005
U. t. trifolii-repentis	22·70±0·10	20·02±0·07	1·14±0·006
U. t. (glomerati)	22·80±0·10	19·42±0·09	1·18±0·007
U. t. fallens	23·39±0·09	21·42±0·06	1·08±0·004

TABLE 13.

Summary of Differences between Means of Dimensions of Uredospores of Subspecies of U. trifolii.

Subspecies Compared.	Length.		Breadth.		Ratio.	
	Difference in Means in Microns.	Test Statistic.	Difference in Means in Microns.	Test Statistic.	Difference in Means.	Test Statistic.
U. t. subterranei B and *U. t. subterranei* A	0·54±0·14	3·9*	0·03±0·11	0·3	0·02±0·006	3·3*
U. t. subterranei B and *U. t. trifolii-repentis*	0·85±0·13	6·5*	0·26±0·10	2·6*	0·05±0·007	7·1*
U. t. subterranei B. and *U. t. (glomerati)*	0·95±0·13	7·3*	0·86±0·11	7·8*	0·09±0·008	11·3*
U. t. subterranei B and *U. t. fallens*	1·54±0·13	11·8*	1·14±0·09	12·7*	0·01±0·006	1·7
U. t. subterranei A and *U. t. trifolii-repentis*	0·31±0·15	2·1	0·23±0·11	2·1	0·03±0·008	3·8*
U. t. subterranei A and *U. t. (glomerati)*	0·41±0·15	2·7*	0·83±0·12	6·9*	0·07±0·009	7·8*
U. t. subterranei A and *U. t. fallens*	1·00±0·14	7·1*	1·17±0·10	11·7*	0·03±0·006	5·0*
U. t. trifolii-repentis and *U. t. (glomerati)*	0·10±0·14	0·7	0·60±0·11	5·5*	0·04±0·009	4·4*
U. t. trifolii-repentis and *U. t. fallens*	0·69±0·13	5·3*	1·40±0·09	15·6*	0·06±0·007	8·6*
U. t. (glomerati) and *U. t. fallens*	0·59±0·13	4·5*	2·00±0·11	18·2*	0·10±0·008	12·5*

* Significant at P=0·01.

(*c*) Comparison of uredospore and teleutospore dimensions.

Tables 10 and 12 show that inter-subspecies differences in teleutospore dimensions are not correlated to corresponding differences in uredospore dimensions. A similar case was recorded by Waterhouse (1930) for Australian physiologic races of *P. g. tritici*. However, within each subspecies of *U. trifolii*, the average teleutospore is both longer

and narrower than the corresponding average uredospore. This seems to be characteristic of the species.

Differences between the spore dimensions of subspecies of *U. trifolii* are in no instance as great as those found by Levine (1923) between subspecies of *P. graminis*. However, subspecies of *U. trifolii* are differentiated by species within the genus *Trifolium*, whereas subspecies of *P. graminis* are specialized largely to different genera of the Gramineae, and a lower order of morphologic variation in the former species would therefore be anticipated.

Conclusions.

Studies of the rusts occurring on five species of *Trifolium* in Australia have distinguished seven biological and morphological entities, viz.: *U. trifolii trifolii-repentis*, *U. trifolii* (*glomerati*), *U. trifolii fallens*, *U. trifolii subterranei* races A and B, *U. flectens* (*repentis*), *U. flectens* (*fragiferi*). *U. trifolii* and *U. flectens* are recognized clover rust species, but only *U. t. trifolii-repentis* and *U. t. fallens* correspond to previously recognized subspecies.

Since physiologic races of a subspecies of *P. graminis* are differentiated by species and varieties within the host *genus*, comparable biological entities within a subspecies of *U. trifolii* would be differentiated by varieties within the host *species*. Races A and B of *U. t. subterranei* do not differ in reaction on the range of *Trifolium* spp. tested, but are separated by varieties of *T. subterraneum*.

Of the three previously recognized subspecies of *U. trifolii*, subterranean clover rust resembles *U. t. hybridi* most closely. Though material of *U. t. hybridi* has not been available for comparison with *U. t. subterranei*, the two rusts apparently differ in their ability to parasitize *T. pratense*. The recognition of *U. t. subterranei* as a fourth subspecies of *U. trifolii* therefore seems justified.

The Australian rust of *T. glomeratum* clearly belongs to the species *U. trifolii*, and closely resembles *U. t. trifolii-repentis*. Limited glasshouse tests have shown the ability of *U. t. trifolii-repentis* to attack *T. glomeratum*, and the resistance of *T. repens* to *U. t.* (*glomerati*). The rusts also differ markedly in teleutospore dimensions. However, the information available is not yet sufficiently extensive to warrant the recognition of *U. t.* (*glomerati*) as a distinct subspecies.

The short-cycle rusts on *T. repens* and *T. fragiferum* belong to the species *U. flectens*. The teleutospores of *U. f.* (*repentis*) are 0·65 ± 0·18 μ longer than those of *U. f.* (*fragiferi*). While *U. f.* (*repentis*) attacks all strains of *T. repens* and *T. fragiferum* tested, *U. f.* (*fragiferi*) is unable to attack available strains of *T. repens*. The reactions of the rusts correspond on the majority of *Trifolium* spp. tested. Classification of the rusts is difficult at this stage. The species *U. flectens* was originally described by Lagerheim as occurring on *T. repens*; the inability of *U. f.* (*fragiferi*) to attack *T. repens* may therefore warrant separation of the rust as a subspecies, implying a lower order of variation in *U. flectens* than in *U. trifolii*. Alternatively, the two microcyclic rusts may be considered as physiologic races of the rust of their common host, *T. fragiferum*, but since the rusts are differentiated by features of their species host ranges, such a concept of racial difference is not comparable with that proposed for *U. trifolii*. It is felt that the rusts are best considered as distinct subspecies of *U. flectens*, but until more is known no decision can be made.

II. Longevity of Uredospores of Subterranean Clover Rust.

Introduction.

Oversummering of uredospores of *U. t. subterranei* in a viable condition has been described. In view of the apparent importance of uredospores in carrying the rust over from season to season, some information is desirable on the longevity of the spores under controlled conditions of temperature and humidity.

Materials and Methods.

Uredospore inoculum freshly produced under glasshouse conditions was stored in small desiccators, the humidity within each of which was regulated by an H_2SO_4:H_2O

mixture. Three levels of relative humidity were used, 30%, 50% and 70%, corresponding to specific gravities of 1·43, 1·34 and 1·24 of the mixture. Three desiccators, one representing each level of relative humidity, were stored in the absence of light in incubators at the six temperatures shown in Table 14.

Germination tests of the material were made at regular intervals, the most satisfactory germination resulting when the spores were dusted on to the surface of tap water in a syracuse dish. Germinations were made at a temperature of 20–23° C.

Results and Discussion.

The results are given in Table 14. A 100% germination is represented by +++, 50% by ++, and 5% by +. Failure of germination is designated by –.

It can be seen that conditions of low temperature and low humidity (30%) are the most favourable for storage of the uredospore inoculum. At 2° C. and 30% relative

TABLE 14.

Longevity of Uredospores of U. t. subterranei *at Various Combinations of Temperature and Relative Humidity.*

Temperature.	Relative Humidity.	Viability at Progressive Storage Periods.			
		36 Days.	61 Days.	97 Days.	145 Days.
2° C.	30%	+++	+++	+++	+
	50%	+++	+++	+++	–
	70%	+++	+++	+	–
5° C.	30%	+++	+––	+++	–
	50%	+++	+++	+++	–
	70%	+––	+++	+	–
10° C.	30%	–––	+++	+++	–
	50%	+++	+++	+++	–
	70%	+++	+++	–	–
15° C.	30%	––+	+++	+++	–
	50%	+––	––+	+++	–
	70%	+–	–	–	–
20° C.	30%	+++	+++	–	–
	50%	++	+	–	–
	70%	–	–	–	–
25° C.	30%	++	+	–	–
	50%	–	–	–	–
	70%	–	–	–	–

humidity, the maximum period of storage is approximately five months. Though conditions in the field are not directly comparable with those prevailing in a controlled experiment of this nature, it can be concluded that repeated infection of volunteer plants throughout the summer period is necessary for the carry-over of subterranean clover rust.

III. THE INFLUENCE ON RUST DEVELOPMENT OF STRAINS OF RHIZOBIUM TRIFOLII.

Introduction.

Among the several nutrients which influence the disease disposition of host plants, nitrogen occupies a central position. Its influence on the development of plant rusts is well established (Chester, 1946). Attention is immediately drawn to the part played by symbiotic nitrogen-fixing bacteria in conditioning rust susceptibility in legume species. An experiment was therefore designed to determine the extent to which the presence of a bacterial symbiont in the root system of a host plant influences rust development.

Materials and Methods.

Seedlings of three varieties of *T. subterraneum* were raised in 6″ × 1″ specimen tubes stoppered with light cotton wool plugs, on a basic agar medium of the following composition: $CaHPO_4$ 1 gm., K_2HPO_4 0·2 gm., $MgSO_4$ 0·2 gm., NaCl 0·2 gm., $FeCl_3$ 0·1 gm., agar 8 gm., water 1 litre. The pH of the medium was adjusted to 6·5 by the addition of 8 ml. of N/10 NaOH per litre.

The following four treatments were compared:

1. Inoculation with an ineffective strain of *Rhizobium*;*
2. No nitrogen source;
3. Inoculation with an effective strain of *Rhizobium*;*
4. Inorganic nitrogen supplement.

Commercial seed of the three host varieties was sterilized with 1/500 $HgCl_2$ and germinated aseptically, after the fifth washing, on yeast-mannitol-agar. For treatments 1 and 3 a suspension was prepared by "rubbing-up" the growth of the bacterial strain with a few ml. of sterile water. The germinated seeds were then mixed with a little of the suspension under aseptic conditions, and sown on the surface of the basic agar medium at two seeds per tube. In the case of treatment 2, seedlings were grown from sterilized and germinated seeds in the basic agar medium described above. A supplement of 0·05% KNO_3 was used in treatment 4.

Two physiologic races of *U. t. subterranei* were employed. Seedlings were inoculated when the first signs of nitrogen deficiency became apparent in seedlings grown under the conditions of treatment 2. The experiment was replicated three times, so that a sample of six seedlings of each variety was available for the determination of the influence of each treatment on the development of each rust race.

The experiment was conducted under laboratory conditions throughout the period October to December, 1951. Lighting was adequate for healthy seedling growth and for satisfactory rust development. The mean daily temperature ranged from 60 to 70° F. Measurements of pustule diameters were made under the low power objective of the microscope, by means of an ocular micrometer. Care was taken not to disturb the uredosori.

Results.

(*a*) Development of the seedlings.

Seedlings subjected to treatments 1 and 2 began to show symptoms of nitrogen deficiency 27 days after germination; it was at this stage that the seedlings were inoculated with the appropriate leaf rust race. By the time the uredosori reached maximum development the deficiency symptoms were acute; the cotyledons were yellow and withering, the leaflets mottled, and the seedlings stunted. No consistent difference in appearance could be detected between the seedlings of treatments 1 and 2.

Seedlings inoculated with an effective strain of *Rhizobium* made normal growth but showed a faint mottling of the leaflets; those grown with a nitrate supplement maintained a healthy dark-green appearance throughout.

(*b*) Nodulation.

Root nodules induced by the ineffective bacterial strains were typically poorly developed, white in colour, and produced in large numbers scattered throughout the root system. With the effective strains a smaller number of large pigmented nodules was formed, usually aggregated towards the surface of the growth medium.

(*c*) Rust development.

The reactions given by the three host varieties to each rust race under the conditions of each nitrogen treatment are indicated in Table 15, expressed in terms of the approximate mean pustule diameter in millimetres on the lower surfaces of the second seedling leaves. Microscopic measurements of pustule diameter were made for all seedlings of the Mt. Barker variety (Table 16). The maximum diameter of each pustule

* These cultures were made available by Mr. J. M. Vincent, to whom thanks are tendered.

was taken, approximately 50 measurements contributing to each mean. Readings for the varieties Dwalganup and Yarloop were estimated by visual comparison with the Mt. Barker series.

An analysis of variance technique (Snedecor and Cox, 1935) was applied to the data (Table 17). It can be concluded that pustule diameter is significantly influenced by the treatment given the host seedlings, and though the two rust races do not appear to differ inherently in pustule size, they do differ in the magnitude of their response to different treatments (significant interaction)

TABLE 15.

Reactions of Varieties of T. subterraneum *to Races A and B of* U. t. subterranei.

Treatment.	Host Variety.					
	Mt. Barker.		Dwalganup.		Yarloop.	
	Race A.	Race B.	Race A.	Race B.	Race A.	Race B.
1. Ineffective *Rhizobium* ..	0·3*C	0·3	0·3	0·3	;	0·4
2. Non-nitrogen	0·4c	0·4	0·3	0·3	;	0·4
3. Effective *Rhizobium* ..	0·5	0·5	0·4	0·4	;	0·5
4. Inorganic nitrogen ..	0·7	0·5	0·7	0·7	;	0·7

* Approximate mean pustule diameter in millimetres.
"c" Denotes the presence of a halo of slightly chlorotic tissue.
"C" Denotes an advanced chlorotic condition in the tissue surrounding the uredosori.
; Denotes the hypersensitive reaction.

TABLE 16.

Mean Diameters of Uredosori of Races A and B of U. t. subterranei *on Mt. Barker.*

Treatment.	Mean Pustule Diameter in Microns.	
	Race A.	Race B.
1. Ineffective *Rhizobium* { Strain LS9*	332 ± 8	310 ± 10
{ Strain L90	322 ± 19	334 ± 13
2. Non-nitrogen	415 ± 21	446 ± 20
3. Effective *Rhizobium* { Strain L91	554 ± 28	460 ± 31
{ Strain L92	530 ± 20	474 ± 25
4. Inorganic nitrogen	695 ± 23	503 ± 18

* Accession numbers at Faculty of Agriculture, University of Sydney.

In Table 18 are shown differences between the treatment means together with the results of tests of their significance. The between-treatment differences shown by race A on Mt. Barker are all highly significant, while the differences between strains within treatments are non-significant. The corresponding differences for race B are somewhat less marked but a similar pattern is evident in the results. Two important observations are:

1. Rust development is more vigorous on seedlings grown in a non-nitrogen medium than on seedlings grown in the same medium in the presence of an ineffective strain of *Rhizobium*. The difference in vigour appears to be independent of the quantitative response to available nitrogen, since in both cases the seed proteins are the only nitrogen source.

2. Seedlings supplied with inorganic nitrogen promote more vigorous rust growth than those inoculated with an effective strain of *Rhizobium*. However, this may be due to a difference in the level of nitrogen available to the rust under the conditions of the two treatments.

These effects can be seen to vary in degree with the rust race and host variety considered. Of particular interest is the stability of the resistant reaction of Yarloop to rust race A.

TABLE 17.

Analysis of Variance of Mean Pustule Diameters of Races of U. t. subterranei *on Mt. Barker.*

Source of Variation.	Degrees of Freedom.	Sum of Squares.	Mean Square.	*F.*
Between treatments	5	120,066	24,013	7·2*
Between rust races	1	8,587	8,587	2·6
Interaction	5	16,626	3,325	8·0**
Total	11	145,279		
Experimental error	676		416	

* Significant at P=0·05, tested against Interaction.

** Significant at P=0·001, tested against Experimental error.

TABLE 18.

Tests of Significance of Differences between Treatment Means.

Comparison.	Race A.		Race B.	
	Difference in Means in Microns.	Test Statistic.	Difference in Means in Microns.	Test Statistic.
Ineffective *Rhizobia* L89 and L90	10±21	0·5	24±16	1·5
Ineffective *Rhizobium* L89 and Non-nitrogen ..	83±22	3·8*	136±22	6·2*
Ineffective *Rhizobium* L90 and Non-nitrogen ..	93±28	3·3*	112±24	4·7*
Non-nitrogen and Effective *Rhizobium* L91 ..	139±35	4·0*	14±37	0·4
Non-nitrogen and Effective *Rhizobium* L92 ..	115±29	4·0*	28±32	0·9
Effective *Rhizobia* L91 and L92	24±34	0·7	14±40	0·4
Effective *Rhizobium* L91 and Inorganic nitrogen	141±36	3·9*	43±36	1·2
Effective *Rhizobium* L92 and Inorganic nitrogen	165±31	5·3*	29±31	0·9

* Significant at P=0·01.

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Literature Cited.

AITKEN, Y., and DRAKE, F. R., 1941.—Studies of the varieties of subterranean clover. *Proc. Roy. Soc. Victoria*, 53 (NS): 342-393.

ARTHUR, J. C., 1934.—Manual of the Rusts in United States and Canada. Pp. 438. Purdue Res. Found., Lafayette, Ind.

THE *CULEX PIPIENS* GROUP IN SOUTH-EASTERN AUSTRALIA. I.

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(*Communicated by D. J. Lee.*)

(Three Text-figures.)

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Introduction.

The mosquitoes of southern Australia have received little attention in the past, but recently work on them has been stimulated, firstly by the introduction, for the control of rabbits, of a myxoma virus, which is carried principally by mosquitoes, and secondly by the occurrence of a severe outbreak of encephalitis in the Murray Valley in 1950. The types of mosquitoes responsible for the transmission of these diseases are not fully known, but the possibility that the *C. pipiens* group might be involved made a close study of this group necessary.

In south-east Australia the group comprises a *pipiens*-complex of three forms and an additional undescribed species. This paper presents a description of this new species. A full account of the *pipiens* complex will be published later.

Culex globocoxitus, n. sp.

Description of Adult.

Holotype ♂.—The head is clothed with scales of the usual form. The flat scales around the eye-margins are white, the narrow curved scales are pale and the erect ones are whitish medially and black laterally. The proboscis is dark with pale scales on the ventral side. The palpi are longer than the proboscis by only two-thirds of the length of the last segment. They are dark with pale scales on the underside of the first three segments; the third segment is also pale laterally, on its distal third. The hairs on the last two segments are relatively short and sparse; the third segment has only 10 long hairs at its tip (Fig. 1).

The dorsum of the thorax is dark brown; the scutellum is lighter. The scutal scales are goldish, those of the scutellum are lighter. The bristles are black. The pleurae have the usual patches of whitish scales. There are no pre-alar scales on the tip of the sternopleuron.

The abdomen is black scaled above. The first tergite has two patches of black scales on its posterior border. The second to seventh tergites have wide unconstricted basal bands of creamy scales; the eighth is pale with two large round patches of black scales. The venter is creamy, with black scales forming median and lateral patches on the second to seventh sternites, and a posterior border to the eighth.

Genitalia.—The coxites are very broad and swollen, with dense yellowish hairs and a bunch of setae on the inner face (Fig. 2).

Legs.—The coxae are pale. The bristles and scales of the front and mid-coxae are black; those of the hind coxae are pale. The legs are blackish dorsally; the femora are pale ventrally. The tip of the hind tibia has a distinct ochreous spot. The tarsi are not ringed.

The wing scales are blackish. The upper fork-cell is twice the length of its stem. The distance between the cross-veins is twice as long as the posterior cross vein. Wing length, 3·0 mm. The halteres are pale with a dark knob.

Paratypes ♂.—The series of 30 paratype males show the following variations: Length of proboscis: 2·02 to 2·71 mm., mean 2·34 mm. On the tip of the third segment of the palpi there are from 6 to 15 long hairs. There is some variation in the width

of the basal tergal bands. On the sixth and seventh tergites the bands sometimes widen laterally to reach the posterior margin of the segments. The black patches on the eighth tergite are sometimes reduced to a few black scales.

Genitalia.—The coxites are swollen and broad with a bunch of setae on the inner face. The style is sickle-shaped, and very narrow distally. The paraproct is without a basal arm; it bears seven fine hairs. The ventral processes of the mesosome are narrow and bent outwardly. The dorsal processes are stout and pointed, and their tips, which are often slightly divided, are directed towards the tips of the ventral processes. The number of hairs on each lateral lobe of the ninth tergite varies from 3 to 6.

Wing length, 3·0–3·5 mm.

Allotype ♀.—This differs from the holotype as follows: The palpi are dark with some pale scales on the third segment. This segment is blunt and has a vestige of a fourth segment (Fig. 1). The proboscis is pale scaled ventrally to the tip. The legs are darker than in the male and dorsally are almost black. The abdomen is black above with wide creamy basal bands. Constrictions between the bands and lateral spots are present only on the second and third tergites. The lateral spots, which are whitish, have approximately the same width as the bands, except on the seventh segment where they are two-thirds the length of the tergite. The apical margin of the eighth tergite is black-scaled. The venter has yellowish scales, with conspicuous median and lateral patches of black scales on the third to seventh segments.

Wing length, 3·8 mm. The upper fork-cell is 3·6 times as long as its stem. The distance between the cross-veins is twice the length of the posterior cross-vein.

Paratype ♀.—The series of 30 paratype females have the following variations: The black median and lateral patches on the venter may be conspicuous or may be reduced to a few black scales. Wing length 3·7–4·7 mm. The upper fork-cell is 3·5 to 5·6 times the length of its stem. The distance between the cross-veins is 2·0 to 2·8 times as long as the posterior cross-vein.

Types: The holotype male and allotype female were bred from larvae collected at Williamstown, Victoria, 4th December, 1951. A paratype series was bred from larvae collected in the suburbs of Melbourne. The holotype and allotype together with the associated larval and pupal skins, the paratype series with ten individually associated larval and pupal skins are in the collections of the National Museum, Melbourne.

Pupa.—The trumpet is almost cylindrical or slightly widened distally and is 6–7 times as long as broad. The opening is oblique and is about one-fourth the length of the trumpet. Seta O has 6–7 branches, P and R have 2–3 branches each; all are slightly plumose. Seta A has three plumose branches on segment III–VI, four on segment VII, 6–7 on segment VIII. Seta B on III has 5–6 weak branches; on IV has 3–4 branches, longer than the length of the segment; on V and VI has 2–3 branches about one and a half times the length of the segment; on VII has 2–3 weak branches equal to length of segment VIII. Seta C on IV–VII has 4–8 branches. The ratio of the paddle is about 1·5.

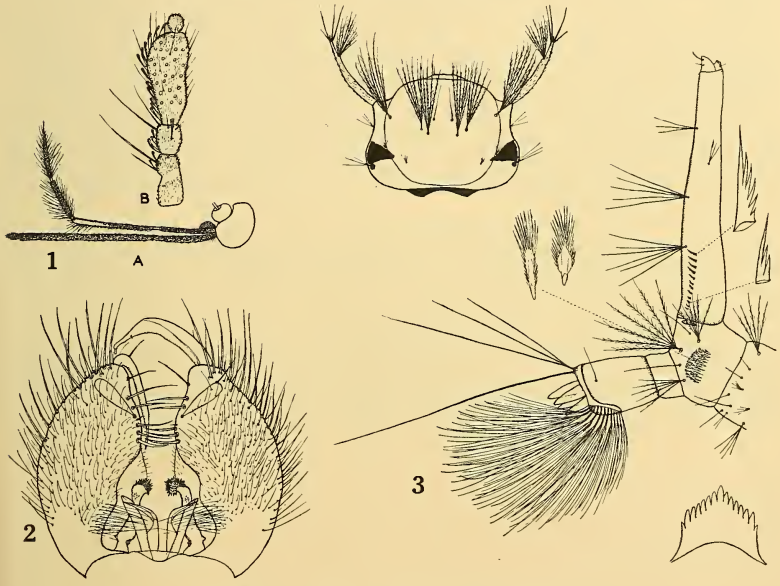
The Fourth Stage Larva (Fig. 3).—The head is yellow. It is one and a half times broader than long. The antennae are brown. They are about five-eighths of the length of the head, and two-thirds of their length from the base bear a tuft of about 23 plumose hairs. The anterior frontal setae are single, the inner frontal consists of five to seven plumose hairs, the mid frontal of three to six and the outer frontal of eight to twelve. The sutural seta (*e*) has three to five branches, the trans-sutural (*f*) four to seven. The mental plate has a large central tooth and seven to eight lateral teeth.

The chaetotaxy of the larval thorax is like that of *C. pipiens* L.; the posterior third of the thorax is sometimes blackish.

Abdomen.—The pentad hairs of the eighth segment: α has 5–7 plumose branches, β is single, γ has 6–9 plumose branches, δ is single and ϵ has 4–6 plumose branches. The comb consists of 44–50 scales arranged in four irregular rows. The dorsal brush usually consists of three inner hairs, rarely four or five, and a single long outer hair. The saddle hair is single. The ventral brush has about twelve tufts. The anal papillae are short, usually one-half to two-thirds the length of the saddle. The siphon is slender

with a very slight sigmoid curve. The siphonal index varies from 4·6 to 6·3, with a mean of 5·5. There are four siphonal tufts on each side. The first consists of three to seven single hairs, the second of three to six, the third of two to four, and the fourth of three to four. The hairs of the first two tufts are about twice the width of the siphon at its base. The third tufts are placed slightly towards the dorsal side. The number of pecten teeth varies from 11 to 15.

The egg-rafts are elongate-oval in shape with 190-302 eggs arranged in 11–14 rows; the number of eggs on the mid-longitudinal line varies from 23 to 31. The index of greatest width to length of the egg is about 0·27. Egg-rafts laid in the laboratory after a human blood meal are small and variable in shape, being triangular, oval or oblong. They contain from 70 to 100 eggs in 5–10 rows, with a median row of 9–17 eggs.



Text-figure 1. Palpi of *Culex globocoxitus*, n. sp.
A, male; B, female.

Text-figure 2. Terminalia of *Culex globocoxitus*, n.sp.

Text-figure 3. *Culex globocoxitus*, n.sp. Head, terminal segments and mentum of larva.

Biology.

This is a stenogamous species; mating will occur in a space as small as three cubic inches. In larger laboratory cages males may mate with resting females, but very commonly the initial coupling occurs when both sexes are in flight.

Reproductive activity is maintained throughout the year (homodynamy). During late July and early August of 1951, pools were found to contain second, third and fourth stage larvae, as well as pupae, from which adults were emerging. In 1952, in the early part of July, pools contained only fourth stage larvae and pupae; in August there were pupae and first stage larvae. It appears therefore that one or two generations are completed during the winter.

Mating would not be inhibited by normal winter temperatures. In the laboratory it has been observed at temperatures down to 13° C.

C. globocoxitus is anautogenous. It is not a man-biting mosquito. On many occasions females have been collected in a bedroom but were never caught freshly engorged. The reduction in the size of the egg rafts laid in the laboratory after engorgement with human blood also indicates that man is not a normal host. The species is probably ornithophilous.

Breeding habitat: Larvae are found in swamps, large and small pools in creek beds and in drainage pits. They will tolerate very polluted water. During the winter the larvae are found in small grassy pools together with *Aë. camptorhynchus* Tomson.

Distribution.—C. globocoxitus occurs throughout Victoria, in the neighbouring parts of South Australia and New South Wales, and in Tasmania. In addition to the type series from suburbs of Melbourne, specimens have been examined from Victoria: Yarram 1♀ 9.4.52, Ararat 1♂ 1.2.52, Ellangerin 1♂ and 1♀ 6.3.52, Lang Lang 2♀ 25.3.52, Cape Paterson 2♂ and 8♀ 5.4.52, Warrnambool 5♂ and 15♀ 29.1.52 and 13–14.2.52 (G. W. Douglas), Inglewood 2♂ and 1♀ 22.4.52, Merbein 2♂ 19.4.52; N.S.W.: Wentworth 1♂ and 1♀ 19.4.52 (N. V. Dobrotworsky); South Australia: Upper south east, nine localities, 4♂ and 6♀ April, 1952 (E. W. L. Lines), 22♂ and 26♀ 19.11–21.12.51, 9–28.2.52; Tasmania: Middleton 1♀ 12.5.48 (E. G. Cannah), Launceston 2♀ 29.3.52, Bothell 1♂ and 1♀ 30.3.52.

Note: *C. globocoxitus* is a member of *Culex pipiens* group but is readily distinguished from the other Australian members. The male can be recognized by the short palpi and by the swollen coxites. The distinctive features of the female are the vestigial fourth segment of palp, the pale scales on the underside of the proboscis and the broad creamy unconstricted tergal bands.

It may be noted that *C. globocoxitus* will inter-breed in the laboratory with the other members of the *pipiens* group in Victoria. In the field three male specimens were obtained whose terminalia were indistinguishable from that of a laboratory *globocoxitus* × *molestus* hybrid.

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